THE SIGNIFICANCE OF CHROMOSOME NUMBERS IN FERNS[1]

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ABSTRACT

The importance of chromosome numbers in ferns is assessed. Some of the distinctive basic numbers ($x = 29$, 37, 39, 41 etc.) give unity to their respective genera. Evolutionary studies should be made in conjunction with geographical studies, and karyotype studies have been undertaken with the promise of advances from some new techniques. The synthesis of many different lines of evidence from many disciplines remains the best hope of achieving the goal of an evolutionary classification.

Any discussion about the significance of chromosomes in the study of pteridophytes starts with Manton's book of 1950. This book was large enough, inclusive enough, critical enough, and dogmatic enough to have had a tremendous impact on the cytogenetics of the pteridophytes. It represents a bench mark as far as the study of fern chromosomes is concerned. It is worthwhile to note that Manton had worked previously on the Cruciferae and thus approached the ferns with the training and bias of a professional cytogeneticist of higher plants. My background and bias is similar, and accordingly I accept the tenets of cytogenetics whether the organism is a moss, an insect, man, or a fern. Chromsomes stain similarly, look similarly, and behave similarly in a broad spectrum of plants and animals. I stress this point, because one should not look for bizarre attributes of chromosomes in the pteridophytes. Chromosomes as we know them must have a long history, and although one can find scholarly works discussing whether the basic chromosome number of the angiosperms was 7 for the primitive woody members or possibly 8 or 6 for the angiosperms as a whole, one cannot find references to where those chromosomes came from or indeed how a chromosome has evolved. Indeed, the molecular biologists are busy building models of chromosomes today which will package perhaps one meter of expanded DNA double helix into "sausages" of 5–10 microns. Undoubtedly, the most important part of the chromosome for its kinetics is the centromere or kinetochore, and so there has been much speculation as to the structure and origin of this region of the chromosome.

However, the cytotaxonomist accepts chromosomes as they are, or as they appear under the light microscope, as valuable aids for the understanding of species relationships. I do not want to present the old debate of classical taxonomy on one side ranged against modern biosystematics on the other. This debate has been well assessed by Heslop-Harrison (1953) and Bennett (1964), and my side of the debate has been well championed by Darlington (1956), Löve (1964), Stebbins (1971), and Grant (1971) among others. Nevertheless, it should be obvious to impartial observers that the controversy continues at

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the working level today. There are still monographs and floras being published in which chromosome numbers may or may not be listed. If they are listed, the information is sometimes added much as one would characterize a species as either annual or perennial. One still sees the statement that "chromosome number is just another character," which is apt to send the cytotaxonomist to the medicine cabinet for another tranquilizer! There is of course sufficient variation in nature, and with the evolution of many bizarre systems antagonists of the biosystematic approach can find much "grist for the mill." Examples that come to mind are the complex heterozygotes of *Oenothera*, unipolar spindles in the fly *Sciara*, and *Claytonia virginica* with recorded chromosome numbers of $2n = 12$ to $2n = 190$ with aneuploid increments of not necessarily even numbers. Perhaps one should mention that star member of the Pteridophyta, *Ophioglossum reticulatum* with $n =$ ca. 630 and $2n = 1260$. Critics are immediately apt to say what difference does a chromosome make with such a superfluity as this?

Although the cytogeneticist considers that chromosomes *are* important and that a karyotype is a visual representation of the blueprint of the plant, he accepts the evidence of the molecular biologist that much of the DNA he is observing is redundant or nonsense DNA. He also accepts the facts that even given the same chromosome number, the amount of DNA can vary markedly as shown in *Vicia*, with DNA values from 17 to 100 (Martin, 1968) or *Pinus* 75 to 139 Miksche, 1967). Admitting that the DNA amounts are variable and that much of this DNA is expendable we are still able to make great use of chromosomes.

If one considers that the first extensive, accurate list of chromosome numbers was in Manton (1950), then lists have appeared regularly since (Chiarugi, 1960; Fabbri, 1963, 1965; Ornduff, 1967, 1968, 1969; Moore, 1970, 1971, 1972). In the two decades post Manton (1950) the chromosome numbers of pteridophytes have become rather well sampled. Indeed, other than those in South America and the Chinese mainland, a broad cross-section of genera and species of the world have been examined. Walker (1972) estimated that 60% of say 300 plus genera have been sampled, but perhaps only 15% of say 12,000 species.

What have these chromosome numbers told us? The first impression one gets is of high numbers. Polyploidy has been a common phenomenon in the history of the present day ferns. This is perhaps not unexpected if we are looking at the last remnants of long lines of descent. In fact we should be impressed that three northeastern North American species of *Osmunda* are uniformly $n = 22$ and have had apparently this chromosome number for perhaps 200 million years (Klekowski, 1970).

We should also be impressed by the stability of some chromosome numbers in spite of their large number and in spite of redundant DNA. Gametic numbers occur such as 36 in *Asplenium*, 37 in *Polypodium*, 41 in *Dryopteris*, and 69 in *Cyathea*, for example.

Fern cytotaxonomists are fortunate indeed to have such distinctive chromosome numbers with which to work and speculate. A gametic number such as 12 as found in *Pinus*, *Solanum*, and *Lilium* does not impart much information regarding the inter-relationships of these genera. It is a further hindrance when one attempts to derive a basic chromosome number. For example, in tomatoes

is $x = 12$, or $x = 6$? Even $x = 6$ is subject to division by two, so that a few might base the series on a palaeobasic $x = 3$. Stable chromosome numbers such as 29, 37 and 41 in ferns are not quite as susceptible to arithmetic manipulations.

The most worthwhile attribute of these numbers is to give unity to a genus. It is intellectually satisfying to find that a genus such as *Dryopteris* s. str., with a common ground plan or phenotype, has also a common basic chromosome number, $x = 41$. With this simple fact, one can then debate the position of the over-named taxa which I will simply refer to as the Oak and Beech ferns. Undoubtedly, it does not work for all genera, but it is extremely useful nevertheless. Even if it is not a solution when one is faced with $n = 29$ or $n = 30$ in *Cheilanthes*, it can be a point of departure for a further examination of the taxonomy of the genus.

Wilce (1972) presents the conservative taxonomic view for the retention of the genus *Lycopodium* with the statement, "I cannot consider a difference in chromosome number sufficient basis for any genus." Also, "to leave *Lycopodium* whole is to maintain a genus that anyone can recognize at a glance, an attribute not to be discarded lightly." Was this not true at one time for the genus *Polypodium*? I would think that one of the strongest cases against such an arrangement is the attempt to treat different genera somewhat equally, or is this being idealistic? If one recognizes segregate genera such as *Aspidotis*, *Phyllitis*, and *Camptosorus* on grounds other than the chromosomal evidence, then surely one is forced to conclude that gametic numbers of 136, 132, 78, 34, 24, and 23, which indicate very ancient dichotomies, should also receive some recognition.

But you may say, what about *Thelypteris* where basic chromosome numbers are known from $x = 27$ to $x = 36$ in an almost unbroken sequence? Smith (1971) has shown for 25 species in the section *Cyclosorus* at least, that x is uniformly 36. It would seem that with further comparative studies of species that much of the seeming diversity might be resolved.

As for higher plants so for ferns, the decision as to whether for example *Dryopteris intermedia* and *D. maderensis* are in fact conspecific rests on whether or not the two species can freely interbreed to give fertile offspring. One critical step in this procedure for the cytogeneticist is whether one sees 41 bivalents with normal pairing and normal crossing-over or not, in the F_1 hybrid. Crosses such as this are difficult, or at least demanding, and have not even been attempted as yet in genera such as *Botrychium* due to the technical difficulties of germinating the spores. However, the recent success of Whittier (1972) would suggest that a crossing program might be possible. A program involving *Botrychium multifidum*, *B. dissectum*, *B. obliquum*, *B. oneidense*, and *B. ternatum* would certainly help to resolve the problem as to whether one should recognize one species or up to five species in northeastern North America (Wagner, 1960).

The cytotaxonomist experiences quite a thrill in uncovering hidden variability at the chromosomal level. For example in *Asplenium trichomanes* (Britton, 1953) and *Pellaea glabella* it was unexpected on morphological grounds that cytotypes of $2x$ and $4x$ would be found. In Table 1, I have shown some paired species from northeastern North America. These are clear examples of a $2x$

TABLE 1. Diploid and tetraploid taxa pairs in eastern North America.

2X	4X
Botrychium lunaria	*B. minganense*
B. lanceolatum	*B. matricariaefolium*
Woodsia ilvensis	*W. alpina*
W. oregana	*W. cathcartiana*
Cystopteris protrusa	*C. fragilis*
Dryopteris intermedia	*D. spinulosa*
D. assimilis	*D. campyloptera*
D. goldiana	*D. celsa*
Asplenium trichomanes	*A. trichomanes*
Pellaea glabella	*P. glabella*
Polypodium virginianum	*P. virginianum*

and 4*x* situation without aneuploidy. I have not attempted in this table to decide which of the tetraploids might be considered autotetraploids, segmental allotetraploids, or genomic alloploids. It is probable that we have a complete range of these conditions represented in the table. The criterion for deciding on homology has been chromosomal pairing. Wagner (1971) has discussed some of the difficulties in interpreting bivalents and univalents in *Dryopteris*, and recently Klekowski (1973) has raised the issue of homologous versus homoeologous pairing. One should not forget that if one is to emulate the models of genomic allopolyploidy as found in cotton, tobacco, oats, and wheat, that the essential proof of the scheme rests on the artificial resynthesis of the species. We have yet to achieve this level of sophistication with ferns except in a couple of instances. The cytogeneticist before studying the chromosomes is unable to predict which species and genera will be uniform in chromosome number and which will show variation. As mentioned before *P. glabella* and *A. trichomanes* show variation in ploidy as does *Dryopteris assimilis* (2*x*) when compared with *D. campyloptera* (4*x*). Here we have little morphological variation and yet polyploidy. Conversely, we may be confronted with a great deal of morphological variation as in *Pteridium aquilinum*, *Athyrium filix-femina*, and *Botrychium dissectum* and yet find cytological uniformity. The situation is not peculiar to our flora, as it has been commented on by Walker (1966) for Jamaica.

Returning to the flora of northeastern North America, one finds fewer clear-cut examples of 4*x* and 6*x* situations than one finds in the tropics. Two reasonably clear-cut examples are shown in Table 2.

Often one has to look outside the floristic region for the related species (Table 3). This is another *major* contribution of chromosome studies, I feel. It forces workers to think of related species and their evolution and to be less provincial

TABLE 2. Tetraploid and hexaploid taxa pairs in eastern North America.

4X	6X
Dryopteris cristata	*D. clintoniana*
Cystopteris fragilis	*C. laurentiana*

TABLE 3. Diploid and tetraploid pairs with one member absent from northeastern North America.

2X	4X
Europe	*Phyllitis scolopendrium*
Europe	*Asplenium ruta-muraria*
Western North America	*Gymnocarpium dryopteris*
Dryopteris abbreviata (Europe)	*D. filix-mas*
C. acrostichoides	*Cryptogramma crispa* (Europe)

in their outlook. The cytogenetic approach can then join hands with plant geography (Britton & Soper, 1966) and consider the various entities on a world-wide basis (*e.g.* Tryon, 1969; Hultén, 1958, 1962).

Vida (1972) says that there are 85 species of ferns in *Flora Europaea*, and then he goes on to present some speculative charts for the evolution of species within *Polypodium*, *Polystichum*, *Dryopteris*, *Asplenium*, *Cheilanthes*, and *Cystopteris*. These genera also occur in our flora, where Fernald (1950) has described 28 genera and 83 species of ferns. Accordingly, the list of the more exciting genera for evolutionary schemes would include the European ones as well as *Botrychium*, *Woodsia*, *Pellaea*, *Gymnocarpium*, and *Phegopteris*.

A further contribution of cytogenetics has been in the study of hybrids. I think we have largely dispelled the concept of F_1s, backcrosses, and F_2 segregates as far as species crosses are concerned. The meiotic irregularities and the aborted spores of such plants as × *D. triploidea* Wherry (*Dryopteris intermedia* × *spinulosa*) would seem to indicate that these plants are evolutionary dead-ends. Also, even after acknowledging what seems like an endless enumeration of such hybrids as have been found or synthesized in the European Aspleniums, *e.g.* diploid *Asplenium trichomanes* × various other species, then again tetraploid *A. trichomanes* × the same species of *Asplenium*, one is still left with a very finite number of possible combinations. For example with *Dryopteris* in a given swamp in southern Ontario, if one finds *D. intermedia* (2*x*), *D. spinulosa* (4*x*), *D. cristata* (4*x*), and *D. clintoniana* (6*x*), it is quite probable that one could find six hybrids involving these four entities. A checkerboard fan would arrive at this by visualizing a 4×4 table yielding 16 combinations (Britton, 1965). He would subtract 4 for selfs giving 12 combinations and then divide by two for reciprocal crosses. Answer—6 hybrids. A non-checkerboard fan might say the first species could cross with each of the other three, the second with each of the other two and the third with the last one giving 3 plus 2 plus 1 interspecific combinations. Unless one is to admit the presence of an undetected other species in the swamp or at its edge, *e.g. D. marginalis* or *D. goldiana*, or decide that spores have been blown in, then six hybrids should be the magic number. In my discipline, this has more reality than trying to match a given plant with some aberrant or monstrous type such as *Polypodium amorphum*, whose chromosomes are unknown and which has never been recollected (Lang, 1969).

The first step in a study of the cytogenetics of a species is the accurate determination of the chromosome number. Unfortunately, this is as far as we have proceeded in many cases, and given the technical difficulties of the material

even this has not been achieved in some cases (Britton, 1964). The second step is to obtain a karyotype. This has not been a popular pastime of cytogeneticists with ferns. Large numbers, small chromosomes, and slowly dividing root-tips are all deterrents, and only the Japanese workers have attempted the painstaking job of comparing karyotypes of different species (Kawakami, 1970, 1971; Takei, 1969; Tatuno & Yoshida, 1966, 1967; Tatuno & Kawakami, 1969; Tatuno & Okada, 1970; Tatuno & Takei, 1969). They have concluded that the present day basic numbers (X) are in fact derived from what they call *b* numbers (palaeobasic numbers?). For example in *Osmunda* where $n = 22$ and x is 22, they consider that b is 11, *i.e.* present-day Osmundas are ancient tetraploids. In *Asplenium* $x = 36$, but they consider that $b = 12$. It is logical to conclude that the present basic numbers in the ferns, which are high in comparison with angiosperm basic numbers, are derived from lower numbers. At the same time, the small size of some of the fern chromosomes and their large numbers make this type of study technically difficult to verify. One might also ask why the chromosomes should be so stable as to have resisted changes such as inversions, translocations, duplications, and deletions which would prevent identifying four or six of each kind of chromosome? New techniques showing fluorescent bands (Vosa, 1971) or Giemsa bands (Evans *et al.*, 1971; Lee *et al.*, 1972) may be of assistance in identifying individual chromosomes. However, the studies of Kurabayashi (1958) on *Trillium* would suggest that polymorphism will present limitations to the use of this technique for precisely identifying each individual chromosome.

I am less enthusiastic about the use of chromosome numbers for grand schemes of phylogenetic relationships. The distinctive numbers such as 37 for *Polypodium* can perhaps be used for evidence of polyphyletic lines, but is this not merely pushing the problems of origin further back into the hands of our paleobotanist friends? Walker (1966) has a phylogenetic scheme for the Hymenophyllaceae based on X numbers of 6, 7, 8, 9, 11, and 13. Each is considered as a different line and we are faced with six origins instead of one! The very fact that we have distinctive gametic numbers such as 29, 37, and 41 makes their inter-relationship and origin obscure. For example in *Marattia* and *Tectaria* the $n = 39$ could have arisen in different ways. As Walker (1966) suggests it may be an example of an aneuploid drop ($n = 40$ to $n = 39$), whereas others have suggested that 39 is three sets of 13. It is unlikely that we will be able to reconstruct the phylogeny of these different numbers. At best, a phylogenetic scheme will not be based on arithmetic manipulations of basic chromosome numbers. Instead, these numbers can be used as ancillary evidence that the scheme offered is not negated by the chromosomal evidence. However, since chromosomal increase by polyploidy and chromosomal decrease by translocations and loss of centromeres are both acceptable to cytogeneticists. I see few stringent restrictions for the speculative phylogenist!

I feel that the best hopes we have for the future are the comparative studies and the synthesis of many different lines of evidence. It is not just the chromosomes, but *all* the available evidence that should be considered. New evidence becomes available all the time. We now have phytochemical studies (Widén

& Britton, 1971 *a*, *b*), SEM studies (Britton, 1972 *a*, *b*), isozyme studies, fluorescent bands on chromosomes, and DNA hybridization studies to mention a few of the newer ones. Tomorrow who knows?

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